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Supplemental Assay Method for the Determination of Residual Moisture in Veterinary Biologics Products

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1. Introduction

Freeze-dried veterinary vaccines always contain some water, commonly known as residual moisture (RM). It is important to determine the level of RM in final products, since a satisfactory test gives assurance of an adequate shelf life and that the manufacturer's freeze-dry cycle was properly controlled. The RM test should confirm that moisture level is consistently within the manufacturer's specification.

This Supplemental Assay Method (SAM) describes the test procedure for residual moisture testing for final product testing of freeze dried new veterinary vaccines.

Residual moisture is determined by the gravimetric method as follows: Residual moisture is driven from the test product by heating under vacuum. The residual moisture content (as per cent) of the test product is calculated based the product weight loss during the drying cycle.

2. Materials

2.1 Equipment

2.1.1 Cylindrical weighing bottles--individually numbered with airtight glass stoppers.

2.1.2 Vacuum oven--equipped with validated thermometer and thermostat. A suitable air-drying device must be attached to the inlet valve.

2.1.3 Balance--capable of readability to 0.1 mg (rated precision ± 0.1 mg).

2.1.4 Desiccator--with phosphorus pentoxide, silica gel or equivalent

3. Preparation for the test

3.1 Personnel training--No specific training is required. Individual should have working knowledge of laboratory equipment listed in **Section 2**.

3.2 Preparation for the test – Environment
Conduct all operations in an environment with a relative humidity less than 45%.

3.3 Preparation for the test – Weighing bottles
Label the weighing bottle for sample(s). Thoroughly clean weighing bottles. Place stopper at an angle on top of bottle and dry for a minimum of 30 minutes at 60°± 3°C under vacuum (<2.5 kPa). While hot, immediately transfer bottles and stoppers into a desiccator. Allow to cool to room temperature, close stopper, weigh and record the weights as "A". Return bottles to desiccator.

3.4 Preparation of the sample - Retain sample, in original airtight containers at room temperature until use. Do not break the seal until ready to proceed.

4. Performance of the test

4.1 Break sample container seal. Using a spatula, break up desiccated product and rapidly transfer (minimum of 100 mg or the amount required for a precise determination at the lower limit, use more than one vial for single dose products if needed) to a previously weighed bottle. Close stopper and immediately weigh. Record the weight as "B".

4.2 Place the bottle with the stopper at an angle in the vacuum oven. Set vacuum to <2.5 kPa and the temperature to 60°± 3°C.

4.3 After a minimum of 3 hrs, turn off the vacuum pump and bleed dry air into the oven until the pressure inside of the oven is equalized with the atmosphere.

4.4 While the bottle is still warm, stopper bottle and transfer to desiccator, and allow to cool to room temperature (for a minimum of two hours or a time validated to yield a constant weight). Weigh, and record the weight as "C".

5. Interpretation of results

Calculate the residual moisture (%) as

% moisture = ((**B** - **C**) / (**B** - **A**)) X (100)

A is tare weight of bottle.
B minus **A** is weight of sample before assay.
B minus **C** is weight equivalent to residual moisture of sample.

6. Report of test results

Test results are reported according to current procedures.

7. References

7.1 Code of Federal Regulations, Title 9, Parts 113.29

7.2 Testing of Residual Moisture, VICH GL26, Final, April, 2002